

**Data Evaluation Report on the Toxicity of Metconazole to Sheepshead Minnow  
(*Cyprinodon variegatus*), Early Life Cycle**  
EPA MRID Number 47795004

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<b>Data Requirement:</b>	EPA DP Barcode	371496
	EPA MRID	47795004
	EPA Guideline	850.1400

**Test material:** Metconazole, technical-grade      **Purity:** 99.4% (84.1% *cis* isomer, 15.3% *trans* isomer)  
**Common name** Metconazole  
**Chemical name:** IUPAC: (1*RS*,5*RS*;1*RS*,5*SR*)-5-(4-chlorobenzyl)-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)-cyclopentanol  
CAS name: 5-[(4-chlorophenyl)methyl]-2,2-dimethyl-1-(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol  
CAS No.: 125116-23-6  
Synonyms: KNF-S-474m

**Primary Reviewer:** Christie E. Padova  
**Staff Scientist, Dynamac Corporation**

**Signature:** *Christie E. Padova*  
**Date:** 02/01/10

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**Date:** 02/12/10

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**Biologist, EPA/OPP/EFED/ERB-1**

**Date:** 12/2/10 *Alicia Korol 1/13/11*

**EPA PC Code** 125619

**Date Evaluation Completed:** 12/2/10

**CITATION:** Lee, M. 2009. Metconazole (KNF-S-474m) – Early Life-Stage Toxicity Test with Sheepshead Minnow (*Cyprinodon variegatus*) Following OPPTS Draft Guideline 850.1400. Unpublished study performed by Springborn Smithers Laboratories, Wareham, MA. Laboratory Study No. 12709.6279. Study sponsored by Valent U.S.A., Walnut Creek, CA. Study initiated December 31, 2008 and completed June 22, 2009.

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**EXECUTIVE SUMMARY:**

The 33-day chronic toxicity of metconazole to the early life-stage of sheepshead minnow (*Cyprinodon variegatus*) was studied under flow-through conditions. Fertilized eggs/embryos (120/level, <30 hours old) of sheepshead minnow were exposed to nominal concentrations of 0 (negative control), 0.94, 1.9, 3.8, 7.5, 15, and 30 µg ai/L (adjusted for purity). Mean-measured concentrations were <0.067 (<LOQ, control), 0.95, 1.5, 3.0, 5.4, 11, and 24 µg ai/L, respectively. The test system was maintained at 23 to 26°C and a pH of 7.6 to 8.0. The 33-day LC<sub>50</sub> was >24 µg ai/L. The 33-day NOAEC and LOAEC values were 11 and 24 µg ai/L, respectively, based on treatment-related reductions in growth (total length and dry weight) at the 24 µg ai/L treatment level.

No treatment-related effects on hatching success (97 to 99% for all levels) or post-hatch survival (95 to 100% for all levels) were observed at any treatment level. The time to hatch, however, was not assessed.

For both total length and dry weight (assessed on Day 33), statistically-significant differences ( $p < 0.05$ ) were observed at the 24 µg ai/L treatment level compared to the control. Total lengths averaged 25.6, 25.7, 25.8, 25.4, 25.5, 25.4, and 24.9 mm and dry weights averaged 0.0687, 0.0699, 0.0716, 0.0707, 0.0728, 0.0684, and 0.0627 g for the control, 0.95, 1.5, 3.0, 5.4, 11, and 24 µg ai/L levels, respectively.

This study is scientifically sound. Due to an omission of the raw data and analysis for time to hatch, this study does not meet guideline standards for an early life stage toxicity study with the saltwater sheepshead minnow, *Cyprinodon variegatus*. **This study is classified Supplemental, but upgradeable.** This study can be upgraded with the submission of the raw data and analyses for time to hatch.

**Results Synopsis**

Test Organism Size/Age(mean Weight or Length): Embryos, <30 hours old  
Test Type (Flow-through, Static, Static Renewal): Flow-through

LOAEC: 24 µg ai/L

NOAEC: 11 µg ai/L

Endpoint(s) Affected: length and dry weight  
Most Sensitive Endpoint(s): length and dry weight

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**I. MATERIALS AND METHODS**

**GUIDELINE FOLLOWED:** U.S. EPA OCSPP (form. OPPTS) 850.1400 (*draft*, 1996)

Deviations included:

1. One result from the nominal 0.94, 1.9, 7.5, and 30 µg ai/L levels exceeded  $\pm 20\%$  of the mean-measured concentration.
2. Water hardness was not monitored during the study. Guidance recommends measuring hardness at all levels at the beginning and end of the test (at a minimum).
3. Raw data pertaining to survival and other clinical effects observed throughout the study and raw data pertaining to test water chemical characteristics (e.g., pH, dissolved oxygen, temperature) were not provided.
4. Time to hatch (*i.e.* number of larvae hatching each day) was not assessed, and as raw daily observation data were not provided, this endpoint could not be visually assessed by the reviewer.

These deviations affect the acceptability of this study.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality claims statements were provided. This study was conducted in accordance with GLP Standards as published in 40 CFR Part 160 with the following exceptions: routine water and food contaminant screening analyses.

**A. MATERIALS:**

**1. Test Material** Metconazole technical-grade

**Description:** Not reported

**Lot No./Batch No. :** AS2122a

**Purity:** 99.4% (84.1% *cis* isomer, 15.3% *trans* isomer)

**Stability of compound under test conditions:** In general, concentrations were satisfactorily maintained within 20% of the mean-measured values for all treatment levels. However, single results were outside  $\pm 20\%$  of the mean-measured concentration for the nominal 0.94, 1.9, 7.5, and 30 µg ai/L levels. Mean-measured concentrations ranged from 72 – 100% of nominal.

**Storage conditions of test chemicals:** In the original container at -10 to -25°C

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**Physicochemical properties of metconazole.**

Parameter	Values	Comments
Water solubility at 20°C	17.1 mg/L cis-isomer 13.6 mg/L trans-isomer	Temp. not reported (source: MRID 46902213)
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

*(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)*

**2. Test organism:**

**Species:**

Sheepshead minnow (*Cyprinodon variegatus*)  
[EPA recommends any of several freshwater fish species, including rainbow trout, brook trout, bluegill, fathead minnow, and channel catfish. See Standard Evaluation Procedure for listing of recommended species. OECD recommends rainbow trout, fathead minnows, zebra fish, and ricefish but does not exclude the use of other species.]

**Age /embryonic stage at test initiation:**

Embryos, <30 hours old  
[EPA recommends fish embryos 2 to 24 hours old.]

**Method of collection of the fertilized eggs:**

Eggs were purchased (assigned SSL Lot No. 09A34). The embryos were acclimated to test temperature (25°C) over a 1-hour period and held in 25°C water until they were placed in the exposure system. Prior to placing the eggs into incubation cups, a sub-sample was microscopically examined for viability; it was reported that the embryos appeared to be healthy.

**Source:**

Aquatic BioSystems, Inc., Fort Collins, CO

**B. STUDY DESIGN:**

**1. Experimental Conditions**

a. Range-finding study: A 19-day (January 8 to 28, 2009) preliminary range-finding study was conducted with 60 sheepshead larvae per treatment level (divided into two replicates) at nominal concentrations of 0 (negative control), 31, 63, 130, 250, and 500 µg ai/L. Following hatch, larvae were thinned to 20 organisms per level. At 14 days post-hatch, growth measurements were recorded.

Hatching success ranged from 85 to 93% for all levels, with no statistically-significant differences from the control observed. Post-hatch larval survival averaged 100, 100, 95, 95, 55, and 10% for the control, 31, 63, 130, 250, and 500 µg ai/L levels, respectively; differences were statistically-reduced ( $p < 0.05$ ; Williams' Test) compared to the control at the 250 and 500 µg ai/L levels. These levels were excluded from further statistical analyses. At 14-day post-hatch, larval lengths averaged 15.6, 14.2, 14.0, 12.7, 9.0, and 8.3 mm for the control, 31, 63, 130, 250, and 500 µg ai/L levels, respectively; differences were

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statistically-reduced ( $p < 0.05$ ; Dunnett's Test) compared to the control at the 31, 63, and 130  $\mu\text{g ai/L}$  levels. Similarly, mean larval dry weights were also statistically-reduced ( $p < 0.05$ ; Dunnett's Test) at the 31, 63, and 130  $\mu\text{g ai/L}$  levels compared to the control. Dry weights averaged 9.31, 6.36, 5.67, 4.08, 1.28, and 0.75 mg for the control, 31, 63, 130, 250, and 500  $\mu\text{g ai/L}$  levels, respectively.

Nominal concentrations used in the definitive study were based on these results and consultation with the Sponsor.

b. Definitive study

**Table 1: Experimental Parameters**

Parameter	Details	Remarks
		Criteria
<u>Parental acclimation, if any</u> Period:  Conditions (same as test or not):  Feeding (type, source, amount given, frequency):  Health: (any mortality observed)	N/A; eggs were purchased	
Number of fertilized eggs/embryos in each treatment at test initiation	120 embryos/treatment level, divided into 30 embryos/cup, 1 cup/aquarium, and 4 replicate aquaria/treatment.	On Day 5, the surviving larvae in each cup were thinned to 10 organisms per replicate (40 per level).  <i>Each treatment should include a minimum of 20 embryos per replicate cup and a minimum of 30 fish per treatment for post-hatch exposure (OECD recommends at least 60 eggs, divided between at least 2 replicates)</i>

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Parameter	Details	Remarks
		Criteria
<p><u>Concentration of test material</u> nominal:</p> <p>mean-measured:</p>	<p>0 (negative control), 0.94, 1.9, 3.8, 7.5, 15, and 30 µg ai/L</p> <p>&lt;0.067 (&lt;LOQ, control), 0.95, 1.5, 3.0, 5.4, 11, and 24 µg ai/L</p>	<p>Water samples were collected from alternating replicate test chambers from all levels on Days 0, 5, 6, 13, 18, 26, and 33.</p> <p>Analytical variation exceeded <math>\pm 20\%</math> of mean-measured values for one measurement per level at the nominal 0.94, 1.9, 7.5, and 30 µg ai/L levels. For these levels, variation was 32, 26, 28, and 27%, respectively (high to low; reviewer-calculated).</p> <p>Mean-measured concentrations ranged from 72 to 100% of nominal concentrations.</p> <hr/> <p><i>A minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate should be used.</i></p> <ul style="list-style-type: none"> <li>- Toxicant concentration should be measured in one tank at each toxicant level every week.</li> <li>- One concentration should adversely affect a life stage and one concentration should not affect any life stage.</li> </ul> <p>OECD recommends that 5 concentrations be spaced by a constant factor not exceeding 3.2; concentrations of test substance in solution should be within <math>\pm 20\%</math> of the mean measured values.</p>
Solvent (type, percentage, if used)	N/A	<hr/> <p><i>The solvent should not exceed 0.1 ml/L in a flow-through system.</i></p> <p><i>Recommended solvents include dimethylformamide, triethylene glycol, methanol, acetone, ethanol.</i></p> <p><i>OECD recommends that the solvent not have an effect on survival nor produce any other adverse effects; concentration should not be greater than 0.1 ml/L.</i></p>

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Parameter	Details	Remarks
		Criteria
<u>Number of replicates</u> control: solvent control: treated ones:	4 N/A 4/level	Number of replicates should be 4 per concentration. A solvent control should be used in conjunction with a solubilizing agent.
<u>Test condition</u> static renewal/flow-through: type of dilution system for flow through method: flow rate: renewal rate for static renewal:	Flow-through Intermittent-flow proportional diluter 7.8 volume additions per day N/A	The exposure system was properly operating for >48 hours prior to test initiation to allow equilibration of the test substance in the diluter apparatus and aquaria.  The diluter system was calibrated prior to test initiation and confirmed at test termination, and the general operation of the diluter was checked visually twice daily during the test. Flow splitting accuracy of the diluter cells was within 5.0% of the nominal value.  Intermittent flow proportional diluters or continuous flow serial diluters should be used. EPA recommends that flow rate to larval cups should provide 90% replacement in 8 to 12 hours (OECD recommends 5 test chamber volumes/24 hours). For static-renewal, OECD recommends 2 renewal procedures; either transfer eggs and larvae to new, clean vessels or retain organisms in vessels and change at least 2/3 test water. A minimum of 5 toxicant concentrations with a dilution factor not greater than 0.5 and controls should be used. Toxicant Mixing: 1) Mixing chamber is preferred; 2) Aeration should not be used for mixing; 3) The test solution should be completely mixed before introduction into the test system; 4) Flow splitting accuracy should be within 10%.

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Parameter	Details	Remarks
		Criteria
Aeration, if any	None reported.	<i>Dilution water should be aerated to ensure DO concentration at or near 100% saturation. Test tanks and embryo cups should not be aerated.</i>
Duration of the test	33 days: 5-day hatching period and 28-day post-hatch period	<i>Recommended test duration is 32 days for EPA. OECD recommendations for test duration are species specific and range from 28-60 days.</i>
Embryo cups, if used type/material (glass/stainless steel):  size:  fill volume:	Round glass jars with 475- $\mu$ m nylon screen bottoms  5-cm diameter, 8-cm high  Not reported	The embryo cups were gently oscillated using a rocker arm apparatus.  <i>Recommended embryo cups are 120 ml glass jars with bottoms replaced with 40 mesh stainless steel or nylon screen.</i>
Test vessel type/material: (glass/stainless steel)  size:  fill volume:	Glass and silicone sealant  30 x 15 x 20 cm, with a 14.5-cm high side drain  6.5 L	<i>Recommended test vessel is all glass or glass with stainless steel frame.</i>
Source of dilution water	Natural sea water was collected from the Cape Cod Canal, Bourne, MA, and diluted to a salinity of $20 \pm 3\%$ with laboratory well water. The diluted seawater had a final salinity of 20 to 24‰ and pH of 7.6 to 8.0 (measured at the in-flow to the diluter system).	The TOC of the dilution water was 1.1 and 0.87 mg/L for the months of March and April 2009, respectively.  <i>Source of dilution water should be natural or reconstituted water; natural water should be sterilized with UV and tested for pesticides, heavy metals, and other possible contaminants. OECD accepts any water in which the test species show control survival at least as good as presented in SEP.</i>

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Parameter	Details	Remarks
		Criteria
<u>Water parameters</u>		
hardness:	Not reported	Hardness and pH range requirements are not reported in OCSPP guidance.
pH:	7.6 to 8.0	
dissolved oxygen:	5.6 to 7.8 mg/L	It was reported that 60% saturation is equivalent to 4.3 mg/L at 26°C and 20‰ and 4.5 mg/L at 24°C and 21‰.
temperature (s) (record all the temperatures used for different life stages):	23 to 26°C	Light intensity ranged from 51 to 88 foot candles (550 to 950 lux) during the study.
photoperiod:	16 hours light/8 hours dark, avoiding sudden transitions	
salinity (for marine or estuarine species):	19.8 to 21.2‰	Raw data were not provided for the water parameters. The salinity range was obtained from the Protocol Deviations section of the study report.
other measurements:	N/A	
interval of water quality measurements:	Temperature, pH, salinity, and DO were measured in each aquarium at test initiation and in alternating replicates daily thereafter. Temperature was also continuously monitored in control replicate vessel A.	<p><i>Recommended hardness: 40-48 mg/L as CaCO<sub>3</sub>;</i></p> <p><i>Recommended pH: 7.2 to 7.6</i></p> <p><i>Dissolved Oxygen (DO) should be measured at each concentration at least once a week;</i></p> <p><i>Freshwater parameters in a control and one concentration should be analyzed once a week.</i></p> <p><i>Temperature depends upon test species and should not deviate by more than 2EC from appropriate temperature.</i></p> <p><i>OECD recommends that DO concentration be between 60 - 90% saturation. As a minimum DO, salinity (if relevant) and temperature should be measured weekly, and pH and hardness at the beginning and end of the test.</i></p> <p><i>Temperature should be measured continuously.</i></p>

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Parameter	Details	Remarks
		Criteria
<u>Post-hatch details</u> when the post-hatch period began:  number of hatched eggs (alevins)/ treatment released to the test chamber:  on what day, the alevins were released from the incubation cups to the test chamber:	Day 5  10 per replicate (40 per level)  Day 5	OCSPP specifies a control hatching success criterion of >75% and a post-hatch survival of 80%. Both validity criteria were met.  <i>Percentage of embryos that produce live  fry should be <math>\geq 50\%</math> in each control;  percentage of hatch in any control  embryo cup should not be more than 1.6  times that in another control cup.</i>
<u>Post-hatch Feeding</u> start date:  type/source of feed:  amount given:  frequency of feeding:	Day 5 (day 0 post-hatch)  Live brine shrimp ( <i>Artemia  salina</i> ) nauplii  <i>Ad libitum</i>  Three times per day	Fish were not fed during the 24 hours prior to study termination.  Exposure aquaria were cleaned when necessary ( $\geq 1$ /week) to remove detritus and excess food.
Stability of chemical in the test system	In general, test levels were maintained within $\pm 20\%$ of mean-measured concentrations. At the nominal 0.94, 1.9, 7.5, and 30 $\mu\text{g ai/L}$ levels, however, the single highest measured concentration (e.g., 1.2 $\mu\text{g ai/L}$ on Day 33 at the nominal 0.94 $\mu\text{g ai/L}$ level) exceeded this range.	
Recovery of chemical:  Frequency of measurement:  LOD: LOQ:	72.3 to 120% of nominal  Days 0, 5, 13, 18, 26, and 33  Not reported 0.055 to 0.067 $\mu\text{g ai/L}$	Based on QC samples spiked (at 0.200, 0.400, 5.00, 30.0, or 300 $\mu\text{g ai/L}$ ), extracted, and analyzed concurrently with each set of test samples.
Positive control {if used, indicate the chemical and concentrations}	N/A	
<u>Fertilization success study, if any</u>  number of eggs used:  on what day the eggs were removed to check the embryonic development:	N/A	

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Parameter	Details	Remarks
		Criteria
Other parameters, if any	The photoperiod was 16-hours light, 8-hours dark, avoiding sudden transitions. The intensity ranged from 51 to 88 footcandles (550 to 950 lux).	

**2. Observations:**

**Table 2: Observations**

Parameters	Details	Remarks
		Criteria
Parameters measured including the sublethal effects/toxicity symptoms	<ul style="list-style-type: none"> <li>- Hatching success</li> <li>- Normal fry at hatch</li> <li>- Larval survival (Day 33)</li> <li>- Measurement of growth (total length and dry weights)</li> <li>- Behavioral and morphological observations</li> </ul>	<i>Recommended parameters measured include:</i> <ul style="list-style-type: none"> <li>- Number of embryos hatched;</li> <li>- Time to hatch;</li> <li>- Mortality of embryos, larvae, and Juveniles;</li> <li>- Time to swim-up (if appropriate);</li> <li>- Measurement of growth;</li> <li>- Incidence of pathological or Histological effects;</li> <li>- Observations of other effects or clinical signs.</li> </ul>
Observation intervals/dates for:  egg mortality: no. of eggs hatched: mortality of fry (e.g., alevins): swim-up behavior: growth measurements: embryonic development: other sublethal effects	Daily Daily Daily N/A Day 33 Not determined Daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Raw daily observation data and chemical characteristics of the dilution water data were not provided.	

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Parameters	Details	Remarks
		Criteria
Other observations, if any	During the 28-day post-hatch exposure period, biomass loading did not exceed 0.051 g/L of flowing test solution per day.	Acceptable under OCSPP guidelines.

## **II. RESULTS AND DISCUSSION**

### **A. MORTALITY:**

No treatment-related effects on hatching success (assessed on Day 5) or post-hatch larval survival (assessed on Day 33) were observed, with no statistically-significant differences from the control indicated for either parameter. Mean hatching success ranged from 97 to 99% for all levels (including the control), and mean larval survival ranged from 95 to 100% for all levels (including the control). The NOAEC for both survival endpoints was 24 µg ai/L.

**Table 3: Effect of Metconazole on egg hatching and survival at different life stage of fish.**

Treatment (µg ai/L) Mean-measured (and nominal) concentrations	Egg hatched/embryo viability			% Juvenile-survival on Day 33 <sup>(c)</sup>
	No. of eggs at study initiation	hatch/embryo viability		
		No. <sup>(a)</sup>	% <sup>(b)</sup>	
Negative control	120	118	99	100
0.95 (0.94)	120	119	99	98
1.5 (1.9)	120	118	98	95
3.0 (3.8)	120	119	99	98
5.4 (7.5)	120	116	97	95
11 (15)	120	118	99	100
24 (30)	120	119	99	98
NOAEC	24 µg ai/L (mean-measured)			24 µg ai/L (mean-measured)
LOAEC	>24 µg ai/L (mean-measured)			>24 µg ai/L (mean-measured)
EC <sub>50</sub>	Not reported			Not reported

<sup>(a)</sup> Reviewer-summed from raw data tables.

<sup>(b)</sup> Percentages were determined per replicate, so the overall mean may not correspond to the total % hatch.

<sup>(c)</sup> Larvae were thinned to 40 per level on Day 5.

### **B. SUB-LETHAL TOXICITY AND OTHER CHRONIC EFFECTS:**

The time to hatch (*i.e.* number of larvae hatching each day) was not evaluated, and raw data were not provided

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for review. A single deformed fry was observed in the control group; otherwise, all hatched fry were normal in appearance for all levels.

A treatment-related effect was observed on growth at the 24 µg ai/L level, as indicated by statistically-significant ( $p < 0.05$ ) reductions in total length and dry weight at this level compared to the control. Total lengths averaged 25.6, 25.7, 25.8, 25.4, 25.5, 25.4, and 24.9 mm and dry weights averaged 0.0687, 0.0699, 0.0716, 0.0707, 0.0728, 0.0684, and 0.0627 g for the control, 0.95, 1.5, 3.0, 5.4, 11, and 24 µg ai/L levels, respectively. The NOAEC level for both growth endpoints was 11 µg ai/L.

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**Table 4: Effect of Metconazole on Growth of Juvenile Fish.**

Treatment ( $\mu\text{g ai/L}$ ) Mean-measured (and nominal) concentrations	Swim-up <sup>(a)</sup>			Length ( $\pm$ SD), mm	Dry weight ( $\pm$ SD), g
	day x1	day x2	day xn		
Negative control	N/A	N/A	N/A	25.6 $\pm$ 0.52	0.0687 $\pm$ 0.0025
0.95 (0.94)	N/A	N/A	N/A	25.7 $\pm$ 0.15	0.0699 $\pm$ 0.0031
1.5 (1.9)	N/A	N/A	N/A	25.8 $\pm$ 0.24	0.0716 $\pm$ 0.0033
3.0 (3.8)	N/A	N/A	N/A	25.4 $\pm$ 0.28	0.0707 $\pm$ 0.0035
5.4 (7.5)	N/A	N/A	N/A	25.5 $\pm$ 0.45	0.0728 $\pm$ 0.0052
11 (15)	N/A	N/A	N/A	25.4 $\pm$ 0.37	0.0684 $\pm$ 0.0046
24 (30)	N/A	N/A	N/A	24.9 $\pm$ 0.42*	0.0627 $\pm$ 0.0031*
NOAEC	N/A	N/A	N/A	11 $\mu\text{g ai/L}$ (mean-measured)	11 $\mu\text{g ai/L}$ (mean-measured)
LOAEC	N/A	N/A	N/A	24 $\mu\text{g ai/L}$ (mean-measured)	24 $\mu\text{g ai/L}$ (mean-measured)
EC <sub>50</sub>	N/A	N/A	N/A	Not reported	Not reported

<sup>(a)</sup> Swim-up is not applicable for this species.

\* Statistically-significant compared to the control data (Williams' Test,  $p < 0.05$ ).

### C. REPORTED STATISTICS:

Data that were statistically analyzed included survival at hatch (hatching success), post-hatch survival (Day 33), and larval growth (total length and dry weight). The time to hatch was not assessed.

Data were evaluated for homogeneity of variance using Bartlett's Test, and for normality using the Shapiro-Wilks Test. The survival data (embryo and larval) did not meet these assumptions and were analyzed by the non-parametric Kruskal-Wallis' Test. The growth endpoints met the assumptions for normality and homogeneity of variance and were analyzed using Williams' Test. All analyses were conducted at the 95% level of certainty except in the case of Shapiro-Wilks' and Bartlett's Tests, in which the 99% level of certainty was applied.

The NOAEC and LOAEC were based on significance data. The MATC was calculated as the geometric mean of the NOAEC and LOAEC. All analyses were performed using TOXSTAT® version 3.5 and mean-measured concentrations.

NOAEC: 11  $\mu\text{g ai/L}$

LOAEC: 24  $\mu\text{g ai/L}$

MATC: 16  $\mu\text{g ai/L}$

Endpoints affected: length and dry weight

Most sensitive endpoint(s): length and dry weight

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**D. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: The reviewer could confirm through visual analysis that there were no effects on embryo hatching success, normal fry at hatch, and larval survival because treatment groups were promoted, unchanged or reduced minimally ( $\leq 5\%$  inhibition) in a non-dose dependent manner, relative to control values. Results for growth parameters were statistically verified using Toxstat statistical software. Data were confirmed to be normally distributed (using the Chi-square and Shapiro Wilks tests) and the variances homogeneous (using Hartley and Bartlett's tests). The NOAEC values for length and weight were determined using ANOVA, followed by Dunnett's (length) and William's tests (weight).

LOAEC: 24  $\mu\text{g ai/L}$

NOAEC: 11  $\mu\text{g ai/L}$

Endpoint(s) Affected: length and dry weight

Most Sensitive Endpoint(s): length and dry weight

**E. STUDY DEFICIENCIES:**

Day of hatch was considered to be exposure day 5, when no more than 10% unhatched viable embryos remained in any control embryo incubation cup. However, time to hatch (*i.e.* numbers of larvae hatching each day) was not assessed and could not be derived by the reviewer because no raw data were provided for this endpoint. The omission of time to hatch places uncertainty in the numbers of larvae hatching each day as well as in the number of viable vs unviable eggs. This omission impacts the study's acceptability.

**F. REVIEWER'S COMMENTS:**

The reviewer's conclusions agreed with those of the study author.

As concentrations were relatively constant throughout the exposure period, the reviewer did not calculate time-weighted average concentrations.

A 1.0-mg ai/L diluter stock solution was prepared by bringing *ca.* 0.0305 g of metconazole (0.0300 g ai) to a final volume of 30 L with dilution water. The solution was mixed for *ca.* 48 hours using a 0.75 hp mixer. The resultant stock solution was clear and colorless. The stock solution was delivered via a FMI pump to a chemical mixing chamber, where it was diluted with 1.97 L/cycle dilution water prior to produce the highest nominal test concentration. This mixture was then proportionally diluted (50%) to produce the remaining nominal test levels. All exposure solutions were clear and colorless throughout the study period.

Water samples were partitioned twice with 50.0 mL of hexane:ethyl acetate (4:1, v:v) per extraction. The combined extracts were reduced to 1 to 2 mL by rotary evaporation, then reduced to near dryness (25  $\mu\text{L}$ ) under a gentle stream of nitrogen. The residues were reconstituted with acetonitrile, vortexed for 30 seconds, and sonicated for 5 minutes. The samples were further diluted with purified water to yield a final composition of 50:50 acetonitrile:water prior to analysis using LC/MS/MS. The method was validated in March 2009. Natural seawater was fortified with metconazole at 0.200, 5.00, or 30.0  $\mu\text{g ai/L}$ . Recoveries averaged  $103 \pm 3.92\%$ , with a LOQ of 0.00938  $\mu\text{g ai/L}$ .

It was determined during pretest analysis that a persistent, low concentration, background level of metconazole was present in the control and blank QC samples. Therefore, all equipment on the exposure system (*i.e.*, exposure vessels, delivery tubing) associated with the controls was replaced with new equipment prior to the

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definitive test. It was subsequently determined that the contamination was likely introduced during sample processing and no metconazole was present in any of the control replicates. Several steps were taken in order to minimize the contamination during the definitive study, including: the processing of control samples was moved to a different area; single-use disposable glassware was used whenever possible, otherwise, acid-washing (initially) or replacement glassware was used and kept segregated for the control samples; all other equipment (i.e., nitrogen concentration, etc.) was replaced; and rinsed vials and additional blank injections were introduced to the LC/MS/MS analysis to minimize carry-over.

Raw data pertaining to test water chemical characteristics (e.g., pH, dissolved oxygen, temperature) were not provided. However, based on acceptable control fish survival and performance, the test media is deemed suitable.

The definitive study was conducted from March 19 to April 22, 2009.

**G. CONCLUSIONS:**

This study is scientifically sound. Due to an omission of the raw data and analysis for time to hatch, this study does not meet guideline standards for an early life stage toxicity study with the saltwater sheepshead minnow, *Cyprinodon variegatus*. **This study is classified Supplemental, but upgradeable.** This study can be upgraded with the submission of the raw data and analyses for time to hatch.

Based on treatment-related effects on growth (total length and dry weight) at the mean-measured 24 µg ai/L treatment level (the only endpoints affected), the NOAEC and LOAEC were 11 and 24 µg ai/L, respectively. No treatment-related effects on hatching success or post-hatch survival were observed.

LOAEC: 24 µg ai/L

NOAEC: 11 µg ai/L

Endpoint(s) Affected: length and dry weight

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**III. REFERENCES:**

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**APPENDIX 1: OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

Total length

File: 50041                      Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	8	12	8	0

-----  
Calculated Chi-Square goodness of fit test statistic = 4.3532

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Total length

File: 50041                      Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

-----  
D = 2.805

W = 0.962

Critical W (P = 0.05) (n = 28) = 0.924

Critical W (P = 0.01) (n = 28) = 0.896

-----  
Data PASS normality test at P=0.01 level. Continue analysis.

Total length

File: 50041                      Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

-----  
Calculated H statistic (max Var/min Var) = 13.13

Closest, conservative, Table H statistic = 216.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 3

Actual values ==> R (# groups) = 7, df (# avg reps-1) = 3.00

-----  
Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal

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but do not differ greatly, the Hartley test may still be used  
as an approximate test (average df are used).

Total length  
File: 50041 Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

-----  
Calculated B statistic = 6.00  
Table Chi-square value = 16.81 (alpha = 0.01)  
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00  
Used for Chi-square table value ==> df (#groups-1) = 6  
-----

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is  
used to calculate the B statistic (see above).

Total length  
File: 50041 Transform: NO TRANSFORMATION

ANOVA TABLE

-----  
SOURCE DF SS MS F  
-----  
Between 6 1.949 0.325 2.425  
Within (Error) 21 2.805 0.134  
-----  
Total 27 4.754  
-----

Critical F value = 2.57 (0.05,6,21)  
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Total length  
File: 50041 Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

-----  
GROUP IDENTIFICATION TRANSFORMED MEAN MEAN CALCULATED IN ORIGINAL UNITS T STAT SIG  
-----  
1 neg control 25.625 25.625  
2 0.95 25.700 25.700 -0.290  
3 1.5 25.800 25.800 -0.676  
-----

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4	3.0	25.400	25.400	0.869
5	5.4	25.500	25.500	0.483
6	11	25.450	25.450	0.676
7	24	24.925	24.925	2.704 *

---

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

Total length  
File: 50041 Transform: NO TRANSFORMATION

DUNNETTS TEST		TABLE 2 OF 2		Ho:Control<Treatment	
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.95	4	0.637	2.5	-0.075
3	1.5	4	0.637	2.5	-0.175
4	3.0	4	0.637	2.5	0.225
5	5.4	4	0.637	2.5	0.125
6	11	4	0.637	2.5	0.175
7	24	4	0.637	2.5	0.700

Total length  
File: 50041 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)				TABLE 1 OF 2	
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	25.625	25.625	25.708
2	0.95	4	25.700	25.700	25.708
3	1.5	4	25.800	25.800	25.708
4	3.0	4	25.400	25.400	25.450
5	5.4	4	25.500	25.500	25.450
6	11	4	25.450	25.450	25.450
7	24	4	24.925	24.925	24.925

Total length  
File: 50041 Transform: NO TRANSFORMATION

WILLIAMS TEST		(Isotonic regression model)		TABLE 2 OF 2	
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	25.708				
0.95	25.708	0.322		1.72	k= 1, v=21

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1.5	25.708	0.322		1.80	k= 2, v=21
3.0	25.450	0.677		1.83	k= 3, v=21
5.4	25.450	0.677		1.84	k= 4, v=21
11	25.450	0.677		1.85	k= 5, v=21
24	24.925	2.709	*	1.85	k= 6, v=21

s = 0.365

Note: df used for table values are approximate when v > 20.

Dry weight

File: 5004w Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	0	11	8	9	0

Calculated Chi-Square goodness of fit test statistic = 7.7946

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Dry weight

File: 5004w Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 2.895

W = 0.967

Critical W (P = 0.05) (n = 28) = 0.924

Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

Dry weight

File: 5004w Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 4.09

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Closest, conservative, Table H statistic = 216.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 7, df (# reps-1) = 3  
Actual values ==> R (# groups) = 7, df (# avg reps-1) = 3.00

---

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Dry weight  
File: 5004w Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

---

Calculated B statistic = 2.00  
Table Chi-square value = 16.81 (alpha = 0.01)  
Table Chi-square value = 12.59 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 3.00  
Used for Chi-square table value ==> df (#groups-1) = 6

---

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Dry weight  
File: 5004w Transform: NO TRANSFORMATION

ANOVA TABLE

---

SOURCE	DF	SS	MS	F
Between	6	2.562	0.427	3.094
Within (Error)	21	2.895	0.138	
Total	27	5.457		

---

Critical F value = 2.57 (0.05,6,21)  
Since F > Critical F REJECT Ho: All groups equal

Dry weight

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File: 5004w Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	6.865	6.865		
2	0.95	6.993	6.993	-0.485	
3	1.5	7.158	7.158	-1.114	
4	3.0	7.070	7.070	-0.780	
5	5.4	7.277	7.277	-1.570	
6	11	6.840	6.840	0.095	
7	24	6.273	6.273	2.256	

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

Dry weight  
File: 5004w Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	4			
2	0.95	4	0.646	9.4	-0.127
3	1.5	4	0.646	9.4	-0.292
4	3.0	4	0.646	9.4	-0.205
5	5.4	4	0.646	9.4	-0.412
6	11	4	0.646	9.4	0.025
7	24	4	0.646	9.4	0.592

Dry weight  
File: 5004w Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2					
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	4	6.865	6.865	7.073
2	0.95	4	6.993	6.993	7.073
3	1.5	4	7.158	7.158	7.073
4	3.0	4	7.070	7.070	7.073
5	5.4	4	7.277	7.277	7.073
6	11	4	6.840	6.840	6.840
7	24	4	6.273	6.273	6.273

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Dry weight

File: 5004w

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)			TABLE 2 OF 2		
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	7.073				
0.95	7.073	0.790		1.72	k= 1, v=21
1.5	7.073	0.790		1.80	k= 2, v=21
3.0	7.073	0.790		1.83	k= 3, v=21
5.4	7.073	0.790		1.84	k= 4, v=21
11	6.840	0.095		1.85	k= 5, v=21
24	6.273	2.257	*	1.85	k= 6, v=21

s = 0.371

Note: df used for table values are approximate when v > 20.